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THE PRODUCTION AND USE OF FLAVOR-POTENTIATING NUCLEOTIDE IN JAPAN

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In 1913 Kodema found that histidine salt of inosinic acid was a principal flavor substance of smoked-dried bonito meats, which is widely employed as a seasoning stock in Japanese cooking and an essential seasoning for preparing consomme type of soup. While mono-sodium plutamate (MSG) was commercially produced few years after it was found to be an essential taste substance in tangle, the production of inosinic acid was long considered not feasible economically. About ten years ago when necessary basic researches aimed at the industrial production of 5'-inosinic acid were initiaded, the first plan was to prepare it from the extractives of dried fish, but was soon abandoned due to the difficulty in obtaining constant supply of fish rich in inosinic acid. Recent advances in chemistry of nucleic acid afforded a great deal of information to the enzymatic decomposition of nucleic acid and the method of identification of decomposition products. This information made it possible to produce inosinic acid on a large scale. On the other hand, the finding that guanylate was about three times as rich as IMP promoted greatly the production of 5'-ribonucleotide by the degradation of yeast ribonucleic acid. A yearly increase in the production of these nucleotides is remarkable. This year, the sixth year of the production, it is expected that about 800 tons of disodium salt of IMP and GMP will be produced.

The application of 5'-ribonucleotides to upgrading the flavor of various foods has been markedly extended. However, the flavor protentiation of fresh meat with 5'-ribonucleotide encountered a difficulty due to its enzymatic decomposition. The object of this paper is to present an information on the production process of 5'-ribonucleotides, flavor quality of foods, and the method of inhibition of enzymatic cleavage of the nucleotides in foods.

METHOD OF PRODUCTION OF 5'-RIBONUCLEOTIDES

Two methods production are now being employed. The first method deals with the decomposition of yeast ribonucleic acid by 5'-phosphodiesterase from microorganisms as shown in Fig. 1. This enzyme is

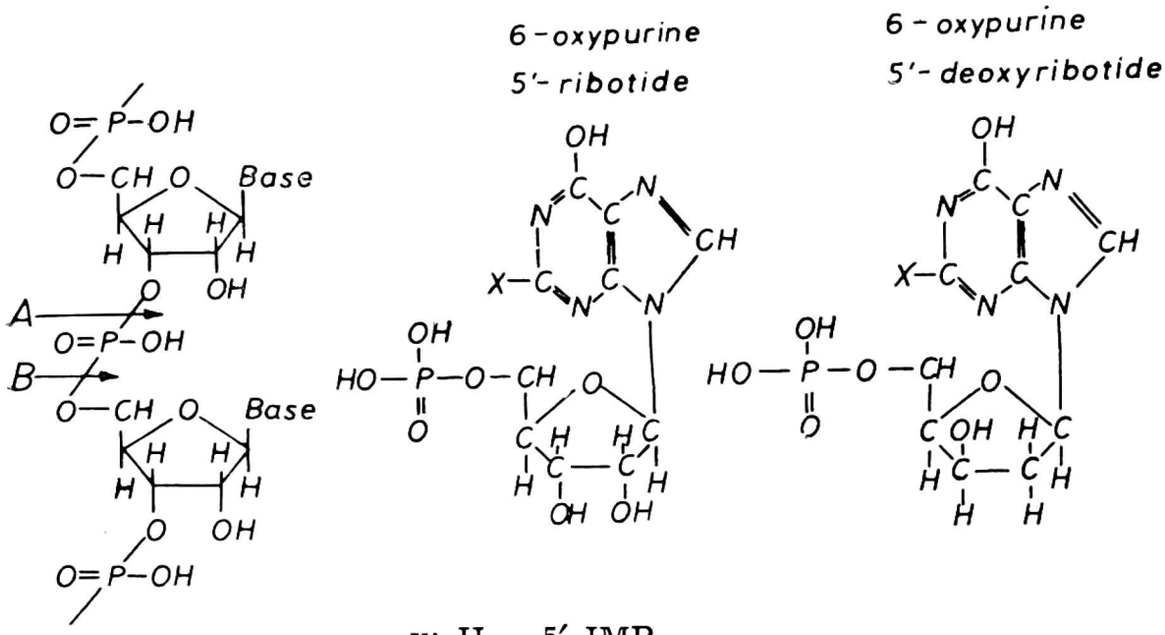

Fig. 1. Enzymes responsible for splitting 5'-and 3'-ester of nucleic acid, and flavor enhancing nucleotides resulted from splitting by 5'-phosphodiesterase

Table 1

Microorganisms excreting 5'-phosphodiesterase from their cells (after Ogata: Chem. & Biol., 1962)

Fungi	*Ascomycetes*	*Aspergillus, Penicillium, Botryosheria, Chaetomidium, Glomerella, Neurospora, Sordairia, Monascus, etc.*
	Fungi imperfecti	*Acrocylindrium, Fusarium, Gliomastix, Helmintosporium, Verticillium, etc.*
Actinomyces		*Streptomyces*
Bacteria		*Bacillus, Clostridium*
Yeast		None

excreted in a submerged culture of microorganisms as given in Table 1. The filtrate of a tank culture of a strain of *St. aureus* is capable of decomposing yeast ribonucleic acid directly into a mixture of IMP, GMP, CMP, and UMP as this strain produces AMP deaminase besides nuclease

and 5′-phosphodiesterase, whereas the filtrate from a culture of *P. citrinum* is devoid of the deaminase, and so it requires the supplement of this enzyme, to produce IMP. An example of the method of production by using *St. aureus* is presented in Fig. 2. The mixture of the nucleotides can be separated through the ion exchange resin.

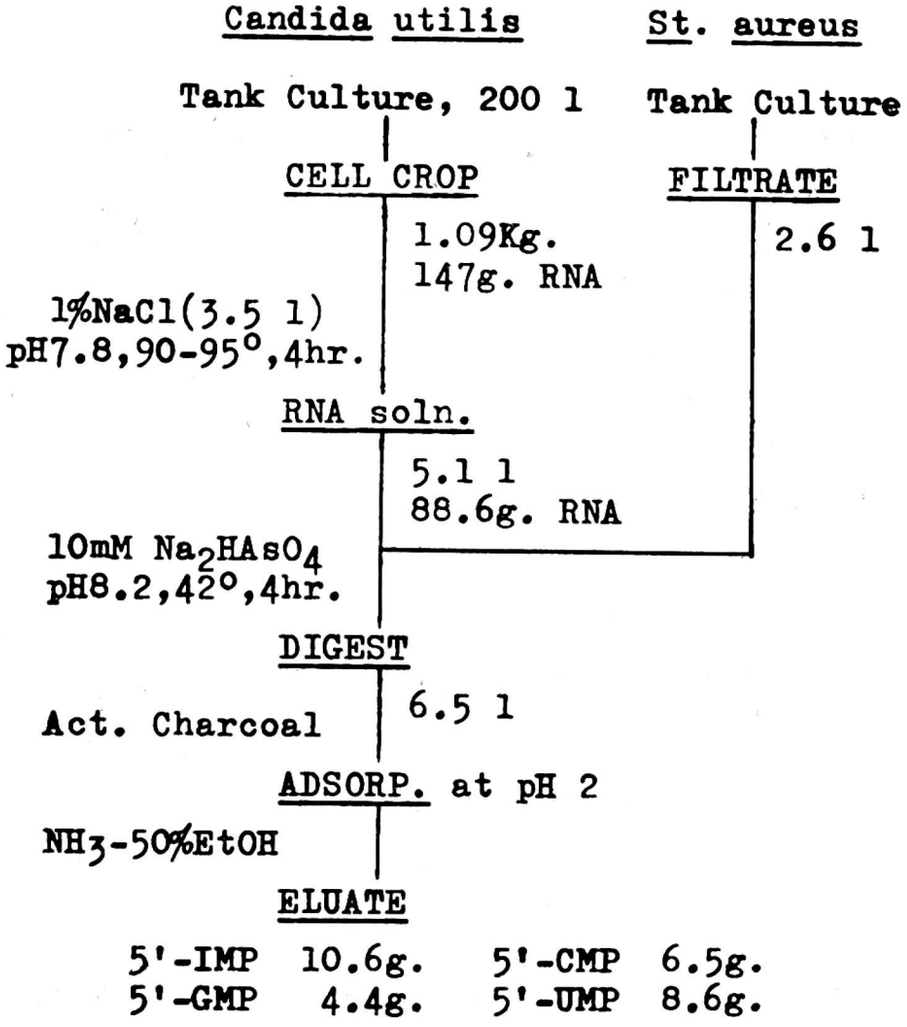

Fig. 2 Preparation of 5'-Nucleotide from Yeast

Fig. 2. Preparation of 5′-Nucleotide from Yeast

The second method involves firstly the biosynthesis of iosine or guanosine from adenine, by an adenine-requiring mutant of *B. subtilis,* followed by chemical phosphorylation at 5' position or by a nucleotide phosphotransforase of a certain strain of bacteria.

Basic studies and pilot tests of various methods based on the autolysis of microbial ribonucleic acid, *de novo* biosynthesis as well as chemical synthesis are.

THE IMPORTANCE OF 5'-RIBONUCLEOTIDE TO THE FLAVOR QUALITY OF FOODS

Among various nucleotides, only those containing a 6-OH at the purine base and a 5'-OH esterified with phosphoric acid at the ribose or deoxyribose possess a strong flaver potentiating ability (Fig. 3) [2]. There is a difference in this potentiating effectiveness between the above essential group containing nucleotides namely, GMP ist about 2.1—5.5 times as much effective as IMP. It is noteworthy that IMP

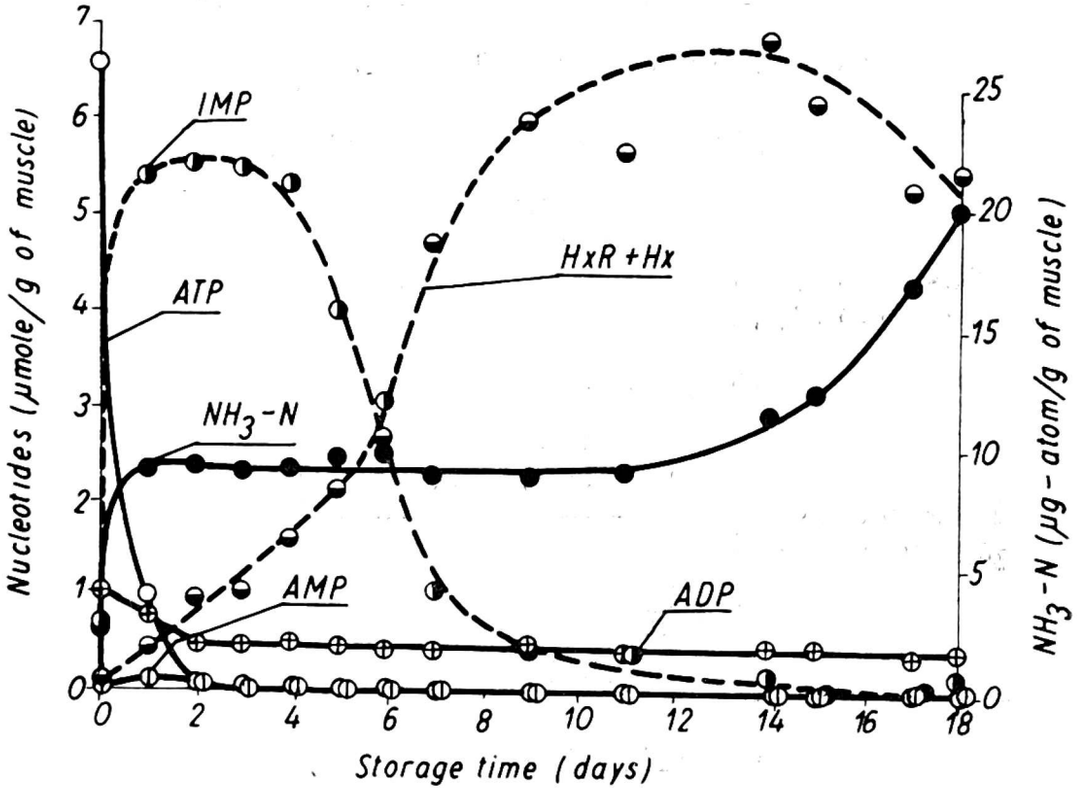

Fig. 3. Change in ATP, ADP, AMP, IMP, Hypoxantine + Inosine, and NH_3-N in carp dorsal muscle during chill-storage

and GMP themselves do not give very strong taste but they give a strong meaty taste when combined with MSG. They also play a role in lowering the bitterness of foods and also in making saltiness harmonized with other tastes of food.

In Japanese cooking, soy sauce and MSG are very popular seasonings so that the content of IMP in fish flesh certainly governs its flavor quality. On the other hand, the flavor quality can not be judged by the content of IMP in such foods that contain little amount of MSG, for example an over-tenderized meat may be preferred although it contains rather a low level of IMP. The flavor in this case may be probably due to that of some autolytic products. Even in this case, however, there is no doubt that the flavor quality can be upgraded markedly by the inclusion of MSG and IMP. Accordingly, it is pertinent to judge the

flavor quality of foods by their content of natural 5'-ribonucleotide. This consideration can be evidenced by studies on the change at 0—4°C in the nucleotides of muscle after sacrifice. Fig. 4 illustrates our data

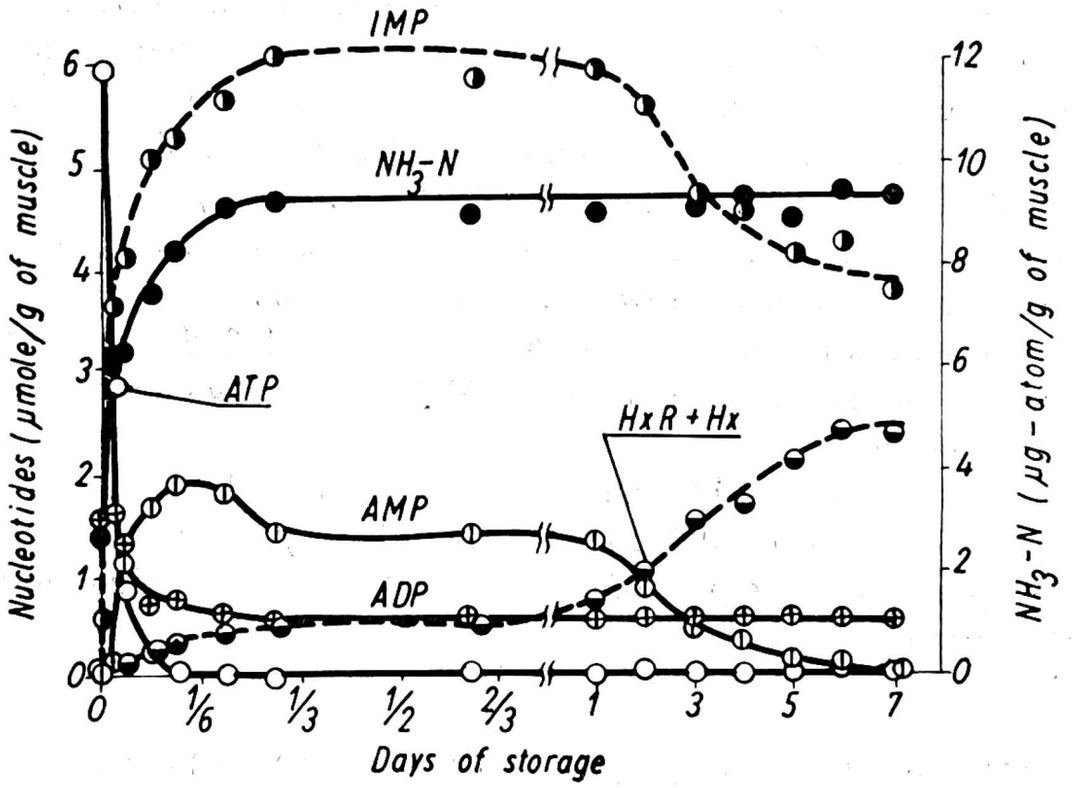

Fig. 4. Change in ATP, ADP, AMP, IMP, Hypoxanthine + Inosine, and NH_3-N in wrasse dorsal muscle during chill-storage

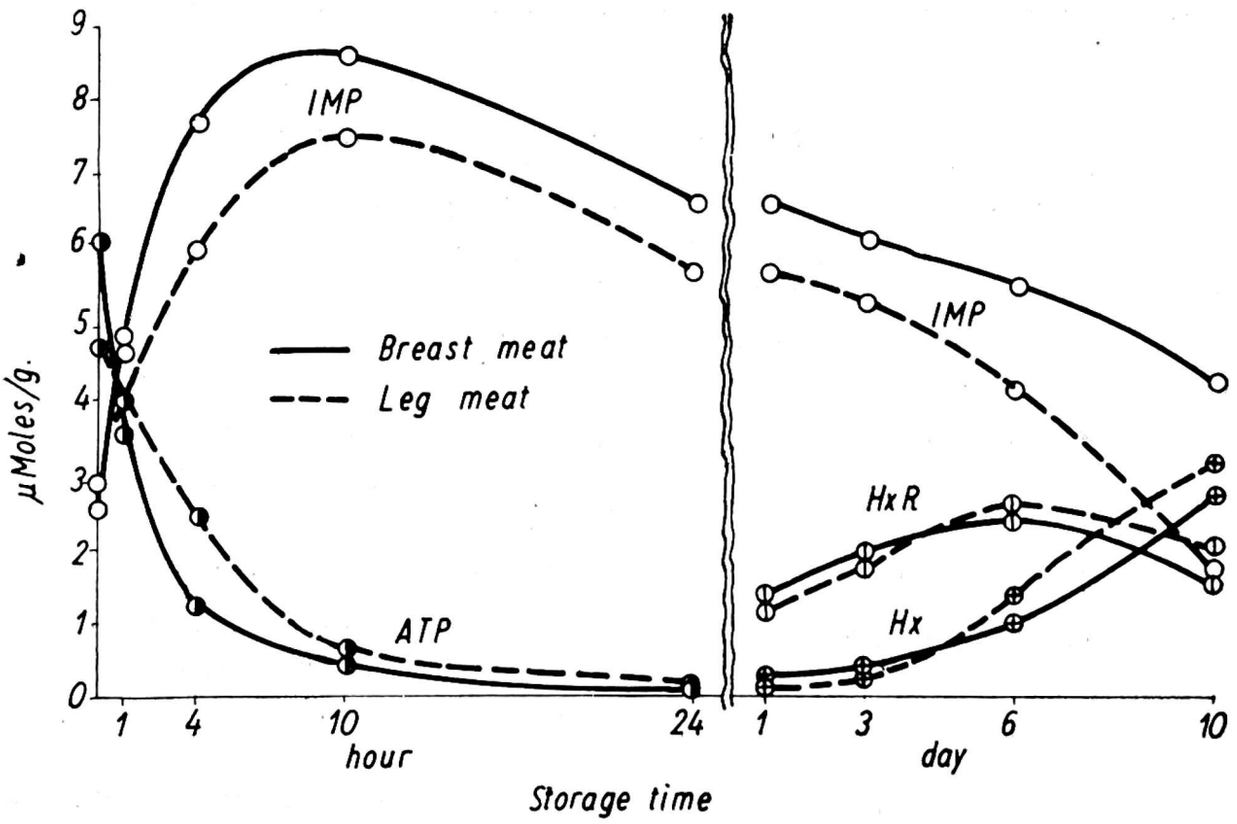

Fig. 5. Change in Concentration of Hx. HxR. IMP and ATP in Chicken Breast and Leg Meats (at 4°C)

on the change in nucleotides of flesh of carp and wrasse, *Pseudolabrus japonicus*, a sea fish stored at 0°C. immediately after slaughter [3]. With an abrupt decrease in ATP, a marked increase in IMP occurred, followed by its gradual decrease. It is to be noted that the amount of ammonia was increased with the production of IMP but remained steady before bacterial spoilage started. The change in nucleotide of chicken and pork meat stored at 4°C. was studied by Terazaki et al.[4] and is shown in Fig. 5. The pattern of change in IMP was nearly identical to that of fish flesh except that the rate of decrease was slower in the meat. A pair test for preference showed that nine panels out of ten preferred the meat with maximum IMP to the meat stored 6 days at 4°C. after slaughter. As the meat with highest IMP content was most preferred, it can be concluded that the content of IMP was intimately related to the flavor and taste of the meat. The foregoing data reveal that the high content of natural IMP does mean not only a good flavor quality due to an appropriate degree of enzymatic change but also safety from bacterial spoilage.

USE OF 5'-RIBONUCLEOTIDE FOR POTENTIATING

Food flavor and some difficulty in its application.

In summarizing recent studies on the distribution of 5'-ribonucleocide in foods, Shimazono [5] reported that the nucleotide distribution patterns

Table 2

Types of Nucleotide Distribution Patterns

1. Meat type	derived from ATP
a) Vetebrate type (meat, fowl, fish)	*IMP*
b) Invertebrate type (shellfish, cuttle fish)	*AMP*
2. Plant type	derived from uridine derivatives, ATP and others. *AMP, UMP.*
3. Milk type	Orotic acid or novel nucleotide
4. Autolytic type	derived from RNA. Autolysate or hot aq. ext. of mushrooms. *GMP*

can be grouped into four types as shown in Table 2. So, the upgrading of the flavor quality can be theoretically achieved by supplementing each type of food with 5'-ribonucleotide (s) which is contained in that

particular type of food. However, among the nucleotides given in Table 2, 5'-IMP and 5'-GMP are stronger flavor potentiators than any other nucleotides. Hence, the use of these two nucleotides in combination with MSG is most effective to enhance the flavor of any type of foods. Two common commercial preparations are sold on the market: one, a mixture of IMP and GMP (1 : 1), e. g., Takeda "ribotide"; the other, a mixture of MEG, "ribotide" (94 : 6), Takeda "EE-CHE-BAN". "Ribotide" is widely employed in food processing, and "EE-CHE-BAN" is very popular in cooking both in restaurants and at home. The kinds of foods which can be upgraded in their flavor from 0.01 to 0,1% by the addition

Table 3

Prevention of hydrolysis of ribotide included in muscle by using coated preparation

Kept 1 hour 40°C	„Ribotide" added mg %	„Ribotide" remained mg %	„Ribotide" remained %
+	30	0	0
—	30	10.5	35
+	50	1.3	3
+	CR 50	36.3	73

„Ribotide": IMP + GMP
CR: coated ribotide

of "ribotide" are soup, sausage, "kamaboko", soy sauce, instant chinese noodles, curry, canned vegetables and so on.

The 5'-ribonucleotide are stable chemically and in cooking, and only partly, at most by 20%, decompose under severe conditions, e. g., autoclaving 1-hour at 120°C. and heating over 100°C. at pH 3. Therefore, none or little destruction of 5'-ribonucleotide occurs when we deal with foods which are heat-processed before and shortly after its addition. But, when 5'-ribonucleotide is to be included in fresh foods such as meat, fermented food, fresh vegetable, etc. it is readily split by non-specific phosphatese and/or 5'-nucleotidase. Therefore, an appropriate method is needed to protect 5'-ribonucleotide from splitting of 5'-terminated phosphate group. The author tried several methods and found two of them can be effectively applied in fresh foods.

The one of the methods studied is to add chelating substances such as polyphosphate, citric acid, etc., to remove certain metals with are required for 5'-nucleotidase activity. A marked effectiveness of this method is shown in Fig. 6. This method can be applied to the preparation of "kamaboko" which is similar to fish ball in the method of

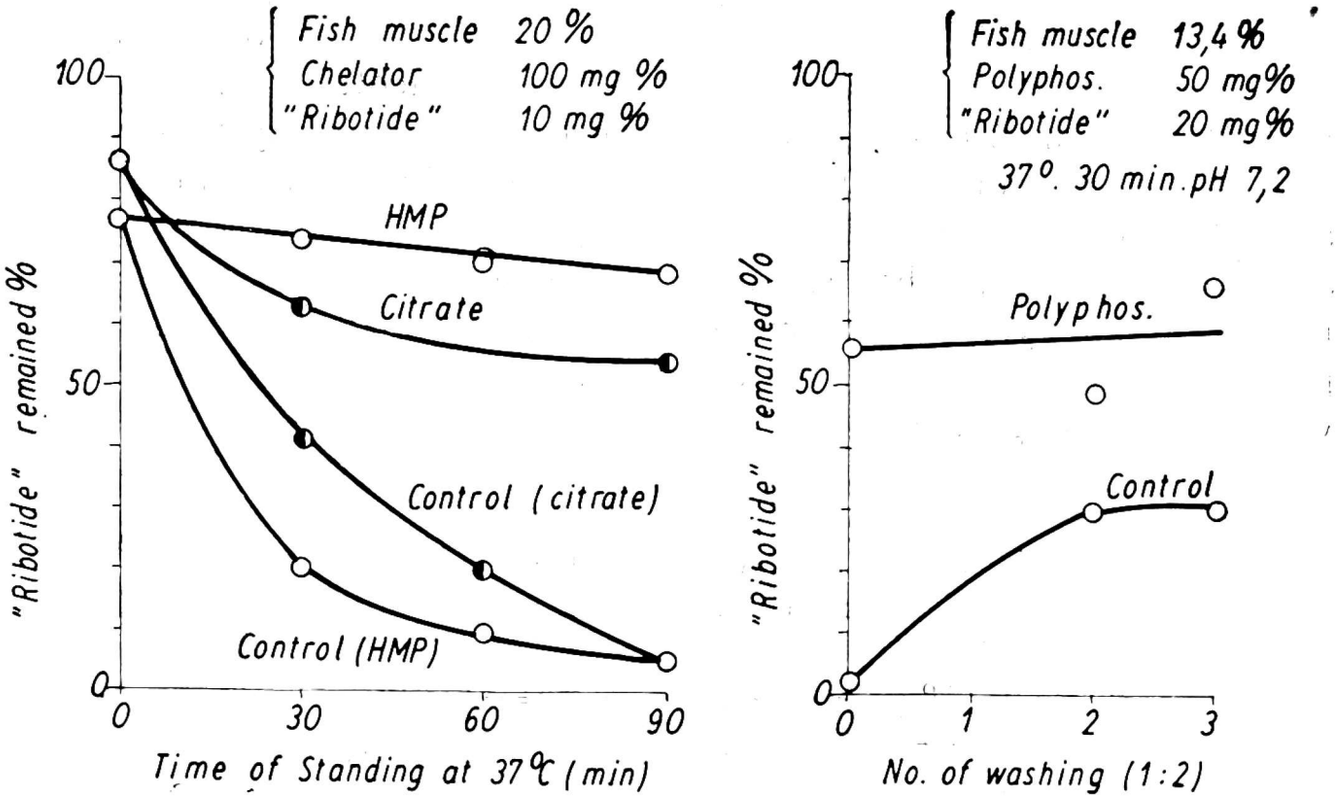

Fig. 6. Effectiveness of chelator to inhibition of enzymatic split of "Ribotide"

preparation. As ground fish flesh rich in 5'-nucleotidase is used for the preparation, the added "ribotide" is otherwise readily decomposed since the pasty flesh is ordinarily kept one hour or so at about 40°C. before cooking.

The other method is to coast the granules of 5'-ribonucleotide with water-insoluble films. This method can be employed for the inclusion of "ribotide" into phosphatase-rich foods. Table 3 indicates a high retension of "ribotide" in "kamaboko" when a coated preparation is used as contrasted to "ribotide" without coating.

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Streszczenie

PRODUKCJA I WYKORZYSTANIE W JAPONII NUKLEOTYDU INTENSYFIKUJĄCEGO SMAK

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Opisano metody otrzymywania w Japonii 5′-rybonukleotydu. Przeprowadzono dyskusję znaczenia 5′-rybonukleotydu dla jakości smaku. Zaprezentowano dwie metody ochrony 5′-rybonukleotydu w środkach spożywczych przed rozkładem enzymatycznym.

Résumé

PRODUCTION ET UTILISATION AU JAPON D'UN NUCLÉOTIDE RENFORÇATEUR DE SAVEUR

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Les méthodes de production de 5′-ribonucléotides au Japan sont décrites. L'importance du 5′-ribonucléotide dans la qualité de la saveur est discutée. On présente deux méthodes pour la protection du 5′-ribonucléotide dans les aliments contre la décomposition enzymatique.

Summary

THE PRODUCTION AND USE OF FLAVOR-POTENTIATING NUCLEOTIDE IN JAPAN

T. TOMIYAMA (FUKUOKA)

The methods the production of 5′-ribonucleotides in Japan are described. The importance of 5′-ribonucleotide to flavor quality is discussed. Two methods are presented for protecting 5′-ribonucleotide in foods from enzymatic decomposition.

Zusammenfassung

HERSTELLUNG UND AUSWERTUNG IN JAPAN EINES DEN GESCHMACK INTENSIVIERENDEN NUCLEOTIDS

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Die in Japan werwendeten Methoden der Herstellung des 5′-Ribonukleotids wurden beschrieben. Die Bedeutung des 5'-Ribonukleotids für die Geschmacksqualität ist diskutiert worden. Zwei Methoden des Schutzes des 5′-Ribonukleotids in Nahrungsmitteln vor dem enzymatischen Abbau wurden angegeben.

Резюме

ПРОИЗВОДСТВО И ИСПОЛЬЗОВАНИЕ В ЯПОНИИ НУКЛЕОТИДА, УСИЛИВАЮЩЕГО ВКУС

Т. ТОМИЯМА (КЮСЮ)

Описаны методы получения в Японии 5'-рибонуклеотида. Проведена дискуссия о значении 5'рибонуклеотида для вкусовых свойств. Приведены два метода защиты 5'-рибонуклеотида в пищевых продуктах от энзиматическоро разложения.